

Application No. 10/729,576  
Response to Office Action Dated February 22, 2006

Attorney Docket No. 60409CON (50370)

**AMENDMENTS TO THE CLAIMS**

The below listing of claims will replace all prior versions, and listings, of claims in the application. Please amend the specification as follows:

1. (Currently amended) A method for identifying a test compound that modulates a heterologous receptor in a cell, said method comprising: providing a cell which comprises a heterologous receptor that is functionally integrated into a signal transduction pathway of said cell, wherein cell surface presentation of a detectable signal comprising a protein product of the AGA2 gene is induced upon activation of said signal transduction pathway; contacting said cell with a test compound; and detecting the level of expression of said detectable signal as a measure of the ability of said compound to modulate signaling via said heterologous receptor.

2. (Original) The method of claim 1, wherein said cell is a yeast cell.

3. (Original) The method of claim 2, wherein said signal transduction pathway is a yeast pheromone response pathway.

4. (Original) The method of claim 3, wherein said cell is a MAT $\alpha$  *Saccharomyces cerevisiae* cell.

5. (Cancelled)

6. (Original) The method of claim 1, wherein said detection step comprises: incubating said cell with a detector molecule conjugated with a reporter moiety, wherein said detector molecule binds specifically to said detectable signal; washing said cell to remove unbound detector molecules; incubating said cell with a substrate appropriate for said reporter moiety; and measuring the readout from said reporter moiety.

7. (Original) The method of claim 6, wherein said detector molecule is the Sag1 protein.

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8. (Original) The method of claim 7, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

9. (Original) The method of claim 6, wherein said reporter moiety is a reporter gene.

10. (Currently amended) The method of claim 6, wherein said reporter gene encodes a polypeptide is selected from the group consisting of beta-lactamase, peroxidase, luciferase, and alkaline phosphatase.

11. (Original) The method of claim 1, wherein said detection step comprises: incubating said cell with a detector molecule conjugated with a reporter moiety, wherein said detector molecule binds specifically to said detectable signal; washing said cell to remove unbound detector molecules; and measuring the readout from said reporter moiety.

12. (Original) The method of claim 11, wherein said detector molecule is the Sag1 protein.

13. (Original) The method of claim 12, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

14. (Original) The method of claim 11, wherein said reporter moiety is a fluorophore.

15. (Original) The method of claim 12, wherein said readout measuring step comprises a fluorescence polarization technique.

16. (Original) The method of claim 1, additionally comprising an extraction step, wherein said cell-surface expressed detectable signal is extracted from the cell prior to said detection step.

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17. (Original) The method of claim 16, wherein said extraction step comprises treatment of said cell with a reducing agent.

18. (Original) The method of claim 17, wherein said detection step comprises: binding of said extracted detectable signal to a support; incubating said support with a detection molecule conjugated with a reporter moiety; and measuring the readout from said reporter moiety.

19. (Currently) The method of claim 18, wherein said support comprises streptavidin-coated scintillation proximity assay SPA beads containing scintillant.

20. (Original) The method of claim 19, wherein binding of said extracted detectable signal to said support is mediated by a biotinylated antibody, wherein said antibody binds specifically to said extracted detectable signal and also to said streptavidin-coated bead.

21. (Original) The method of claim 18, wherein said detection molecule is the Sag1 protein.

22. (Original) The method of claim 21, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

23. (Original) The method of claim 18, wherein said reporter moiety is a radiolabel.

24. (Original) The method of claim 23, wherein said radiolabel is  $^{125}\text{I}$  or  $^3\text{H}$ .

25. (Original) The method of claim 18, wherein said readout measuring step comprises detection of emitted light.

26. (Original) The method of claim 4, wherein said *S. cerevisiae* cell has the endogenous AGA1 gene deleted, such that the AGA2 gene product is secreted.

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27. (Original) The method of claim 26, wherein said detection step comprises: binding of said secreted AGA2 gene product to a support; incubating said support with a detection molecule conjugated with a reporter moiety; and measuring the readout from said reporter moiety.

28. (Original) The method of claim 27, wherein said support comprises streptavidin-coated SPA beads containing scintillant.

29. (Original) The method of claim 28, wherein binding of said secreted Aga2 protein to said support is mediated by a biotinylated antibody, wherein said antibody binds specifically to the secreted Aga2 protein and also to said streptavidin-coated bead.

30. (Original) The method of claim 27, wherein said detection molecule is the Sag1 protein.

31. (Original) The method of claim 30, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

32. (Original) The method of claim 27, wherein said reporter moiety is a radiolabel.

33. (Original) The method of claim 32, wherein said radiolabel is  $^{125}\text{I}$  or  $^3\text{H}$ .

34. (Original) The method of claim 27, wherein said readout detection step comprises detection of emitted light.

35. (Original) The method of claim 2, wherein said heterologous receptor is a G-protein coupled receptor.

36. (Original) The method of claim 2, wherein said heterologous receptor is selected from the group consisting of melatonin receptor 1a, galanin receptor 1, neurotensin receptor,

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adenosine receptor 2a, somatostatin receptor 2, and corticotropin releasing factor receptor 1.

37. (Original) The method of claim 36, wherein said heterologous receptor is melatonin receptor 1a.

38. (Original) The method of claim 35, wherein said heterologous G-protein coupled receptor functionally couples to the endogenous yeast GPA-1 protein subunit.

39. (Withdrawn) A kit for screening of test compounds that modulate a heterologous receptor in a cell, said kit comprising: a cell which comprises a heterologous receptor that is functionally integrated into a signal transduction pathway of said cell, wherein a signal molecule is expressed on the cell surface of said cell upon activation of said signal transduction pathway; and a means for detecting said signal molecule.

40. (Withdrawn) The kit of claim 39, further comprising appropriate buffers and instructional materials for quantitating said signal molecule.

41. (Withdrawn) A kit for screening of test compounds that modulate a heterologous receptor in a cell, said kit comprising: a cell which comprises a heterologous receptor that is functionally integrated into a signal transduction pathway of said cell, wherein a signal molecule is secreted from said cell upon activation of said signal transduction pathway; and a means for detecting said secreted signal molecule.

42. (Withdrawn) The kit of claim 41, further comprising appropriate buffers and instructional materials for quantitating said secreted signal molecule.